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Evaluation of the Deuterium Dilution Method to Estimate Body Composition in the Barnacle Goose: Accuracy and Minimum Equilibration Time

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ABSTRACT

We examined body composition in barnacle geese (*Branta leucopsis*) by proximate carcass analysis and by deuterium isotope dilution. We studied the effect of isotope equilibration time on the accuracy of total body water (TBW) estimates and evaluated models to predict fat-free mass (FFM) and fat mass (FM) from different measurements varying in their level of invasiveness. Deuterium enrichment determined at 45, 90, and 180 min after isotope injection did not differ significantly. At all sampling intervals, isotope dilution spaces (TBW_d) consistently overestimated body water determined by carcass analysis (TBW_c). However, variance in the deviation from actual TBW was higher at the 45-min sampling interval, whereas variability was the same at 90 and 180 min, indicating that 90 min is sufficient time to allow for adequate equilibration. At 90 min equilibration time, deuterium isotope dilution overestimated TBW_c by $7.1\% \pm 2.6\%$ ($P < 0.001$, paired t -test, $n = 20$). This overestimate was consistent over the range of TBW studied, and TBW_c could thus be predicted from TBW_d ($r^2 = 0.976$, $P < 0.001$). Variation in TBW_c and TBW_d explained, respectively, 99% and 98% of the variation in FFM. FM could be predicted with a relative error of ca. 10% from TBW estimates in combination with body mass (BM). In contrast, BM and external body measurements allowed only poor prediction. Abdominal fat fresh mass was highly correlated to total FM and, if the carcass is available, allows simple means of fat prediction without dissecting the entire specimen.

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Introduction

Somatic stores are a key factor in defining an animal's body condition, and much of the variation in survival and reproduction has been attributed to the optimization of body reserves (Blem 1990; Carey 1996; Houston et al. 2007). Because of the central importance of energy and nutrient storage, their assessment has become an increasingly important aspect in current research (Brown 1996; Stevenson and Woods 2006). Birds in particular face the energetic dilemma of high energy expenditure for activity and maintenance and limitations of storage abilities due to their aerial lifestyle (McNab 2002).

A variety of methods have been applied to assess fat mass (FM) and fat-free mass (FFM) in vivo and in vitro (overviews in Blem 1990; Brown 1996; Gessaman 1999; Speakman 2001; Stevenson and Woods 2006). These methods differ in the accuracy of measuring the variable of interest and in the degree of invasiveness for the study animal. Although proximate body composition analysis is regarded as the most accurate method and the standard against which all other methods are evaluated, it obviously represents the most invasive, that is, lethal, avenue. Additionally, it is a labor- and time-intensive method. From such carcass analyses, researchers have recognized that an animal's body water content represents a fairly stable proportion of the FFM because lipids are stored nearly free of water (Pace and Rathbun 1945; Odum et al. 1964; Ellis and Jehl 1991). While some animals can experience large changes in body mass and composition, particularly during extensive periods of fasting, the relative water content in the FFM (H_2O_{FFM}) seems not to be significantly affected (Groscolas et al. 1991; Cherel et al. 1992). Consequently, estimating total body water (TBW) content enables prediction of FFM and subsequently, in combination with total body mass (BM), FM. Indeed, many studies used body water content estimates to successfully predict body stores (Campbell and Leatherland 1980; Miller 1989; overview Table VI in Blem 1990; Boos et al. 2000; but see Jamieson et al. 2006). Using calibrated regressions with dissectible fat depots, such as abdominal and leg fat pads, represents another method to estimate total FM in carcasses without analyzing the entire specimen (Thomas et al. 1983; Piersma 1984; Boos et al. 2000; Jamieson et al. 2006).

The refinement of isotope analysis techniques has yielded nondestructive means to estimate TBW by the principle of isotope dilution, allowing longitudinal studies and work where killing the animal is not an option. This method relies on releasing isotopically labeled water molecules into the body water pool and, after mixing, determining isotope concentrations in body fluids (usually blood) of a single timed sample (plateau approach) or a series of samples (intercept approach) to derive an estimate of TBW (Speakman et al. 2001). Because it involves only one sampling event and a shorter experimental period, the plateau approach is less invasive for the animal. Additionally, a single sample reduces costs for material and analysis. However, with the plateau approach, appropriate timing of the single sample is important, that is, after mixing of the marker with the body water is completed and before wash-out of the marker becomes effective. To reduce the latter, captive animals are deprived of food and water during the equilibrium period. The time a wild animal is held in captivity can crucially affect its performance, in particular during the breeding period when parental care for a clutch or brood has to be provided. Our general research goal is to employ isotope dilution to assess body composition of incubating barnacle geese (*Branta leucopsis*) in the field. To this end, we want to reduce the equilibrium time without affecting data quality. Furthermore, it is known that the isotope dilution method generally overestimates the actual TBW volume because some of the labeled atoms exchange with nonaqueous body constituents (Culebras and Moore 1977; Schoeller et al. 1980). A review of studies employing hydrogen isotopes in four bird species revealed a considerable variation by which the dilution space differed from actual TBW, ranging from underestimates of 2.3% to overestimates of 18% (Table 3 in Speakman et al. 2001). As has been noted frequently (Gessaman 1999; Mata et al. 2006; Shaffer et al. 2006), there is clearly a need for more bird studies evaluating estimates of TBW and other body components measured by isotope dilution against standard proximate body composition analysis.

In this study on captive barnacle geese, we compared estimates of TBW, FFM, and FM measured by proximate carcass analysis and by deuterium isotope dilution. Our specific objectives were to (1) assess how equilibration time may compromise accuracy of the dilution method; (2) determine the level of overestimation of TBW by deuterium dilution; and (3) evaluate the accuracy of predictions of FFM and FM from TBW and/or other predictor variables (BM, external morphological measurements, abdominal fat), depending on lethal and non-lethal approaches.

Methods

Animals and Experimental Setup

Animals were taken from a stock of barnacle geese *Branta leucopsis* kept at the Biological Centre of the University of Groningen in Haren, The Netherlands. Geese were kept on grassland while receiving ad lib. supplementary food (a mixture of

grain and pellets). A total of 21 adult birds (≥ 2 yr old), consisting of 10 females and 11 males, were selected to achieve maximal range in body condition; the body condition criterion was residual BM from a regression of BM on the first principal component from a factor analysis including tarsus and total head length. To further increase the variation in body condition, we kept a subsample of two females and two males separate from the stock on grassland of lower food quality and with only limited supplementary food for 2 wk before the experiment. Their average mass loss during this period was 210 ± 72.5 g. All animals were used in the carcass analysis but only 10 of the 11 males for the isotope dilution space measurements because of leakage of the isotope mixture through the puncture hole of the thin skin in one male. Birds were sexed by cloacal inspection and confirmed by examination of gonads during dissection. Most birds (16) were collected from February to mid-March 2006 and the others (four males, one female) on April 21, 2005.

To standardize treatment, animals were put in bird cages with no access to food but access to drinking water on the evening before the isotope dilution experiment. The next morning (ca. 15 h later), 2 h before administration of the isotope solution, drinking water was removed until the end of the experiment, 4–6 h later. The birds were intraperitoneally injected with a 99.9% deuterium isotope solution (Sigma Chemicals) using 1.0-mL insulin syringes. The actual mass of each volume injected was determined by weighing the syringe before and after injection on an analytical balance (Mettler AG204) to the nearest 0.1 mg. Average dose mass was 1.1153 ± 0.0021 g ($n = 20$). Blood samples were collected from nine females and seven males at each of the following sampling times: 45, 90, and 180 min after injection. Additionally, one female and three male birds were sampled at 90 min. To estimate deuterium background levels, blood samples before isotope administration were taken from three female and three male birds. Blood was collected from the brachial and intertarsal veins and stored in flame-sealed microcapillaries. After the last blood sample was taken, birds were fully anesthetized with 3 mL intraperitoneally injected Nembutal (natriumpentobarbital 60 mg/mL), followed by cervical dislocation 10–15 min later. BM was then measured to the nearest 1 g, and carcasses were placed in plastic bags and refrigerated until being further processed on the next day or were double-packed and frozen at -20°C until dissection and body composition analysis. Daily care and management of the animals and the experimental protocol were approved by the Animal Experimentation Committee of the University of Groningen (license DEC 4081B).

Isotope Analyses

The blood in the capillary tube was distilled in a vacuum line, where water vapor was cryogenically trapped in a quartz tube using liquid nitrogen. After complete transfer, the vacuum system was brought to room pressure by admitting dry nitrogen.

The insert was then quickly brought into a standard vial for automatic injection and sealed with a septum. During the sample preparation, internal water standards (gravimetrically prepared from pure deuterated water and also stored in flame-sealed capillaries) covering the entire enrichment range of the blood samples were distilled alongside the samples. This way, possible systematic effects on isotope enrichment due to the distillation process were accounted for. Such effects were also monitored in every batch by comparing the distilled standard waters with the same waters that were introduced into the vials directly. The actual $\delta^2\text{H}$ measurements were performed in automatic batches using a Hekatech high-temperature pyrolysis unit (Gehre et al. 2004) in which the injected water reacted with the glassy carbon available in the reactor according to $\text{H}_2\text{O} + \text{C} \rightarrow \text{H}_2 + \text{CO}$. The H_2 and CO gas, emerging into a continuous He flow through the system, were then led through a gas chromatography (GC) column to separate the two gases in time and fed into a GVI IsoPrime isotope ratio mass spectrometer for the actual isotope analysis. For the analysis of $\delta^2\text{H}$ (from the H_2 gas emerging first from the GC column), every sample was injected typically six times from the same vial into the furnace in 0.2- μL quantities. Memory effects of the high-temperature pyrolysis oven were corrected for using a memory correction algorithm similar to the one described by Olsen et al. (2006). In the complete analysis scheme, several quality checks were incorporated. The isotope scales were calibrated using multiple distilled samples of two of the standard waters (being at the lower and the higher end of the sample range, respectively), whereas the measured $\delta^2\text{H}$ values for a third standard, representing the expected midrange of blood samples, were used as quality “targets” and had to be measured within 1% of their assigned values in order to meet the quality criterion for the batch. All sample analyses were run at least in duplicate (more times if values differed by more than 2.5%), and we used the average of values differing from each other by less than 2.5%.

Measurement of the Hydrogen Dilution Space (TBW_d)

Using the plateau approach (Speakman et al. 2001) and employing equation (1), we calculated the hydrogen dilution space (TBW_d) by taking into account the quantity of the dose (Q_d , mol), the ^2H concentration of the dose (C_d , atom%), the ^2H background concentration (C_b , atom%), and the ^2H concentration of individual blood samples (C_i , atom%) taken at the various sampling intervals:

$$\text{TBW}_d = 18.02 \times Q_d \times \frac{C_d - C_i}{C_i - C_b}. \quad (1)$$

Background levels of $\delta^2\text{H}$ measured in six birds before dose administration averaged 4.11‰, with a range of 33.01‰, which represented only 0.60‰ of the average measured deuterium enrichment after dose injection (5,532‰, $n = 52$). Therefore, we applied this average background value for all birds.

Dissection and Body Composition Analysis

Fresh or thawed carcasses were weighed; then, all feathers were plucked and the birds reweighed, with the difference being plumage fresh mass. All skin was removed as was associated subcutaneous fat. The following organs were dissected out, weighed, and analyzed for water and fat content: left flight muscle complex (pectoralis and supracoracoideus), left leg musculature (attached to the tibiotarsus and femur), abdominal fat (a discrete deposit in the abdominal cavity, excluding mesenteric fat adhering to the intestines), gizzard, intestines (including mesenteric fat and ceca), heart, liver, spleen, and kidneys. Before analysis, the esophagus, gizzard, and intestines were emptied and reweighed. The total wet content excised from these organs was 20 ± 9 g. The right flight and right leg musculatures were excised and retained for other work, and their contributions to dry mass and FFM were estimated via the equivalent masses of their left counterparts. Organs were cut into small pieces of ca. 1 cm^3 , and bones of the skeleton were broken to expose marrow and brain before being oven-dried at 60°C until constant mass (7–15 d). Total body water from the carcass analyses (TBW_c) was calculated as the carcass fresh mass after plucking minus the sum of all dried tissues, thereby accounting for general water loss during dissection. Thus, water absorbed by feathers is intentionally not included in TBW_c because (external) plumage water is not part of the body water pool measured by isotope dilution. Lipids were extracted from the tissues with a soxhlet apparatus using petroleum ether as a solvent. We refer to whole-body FFM as total wet lean mass, including feathers and skeleton, calculated from fresh BM minus extractable FM.

Calculations and Statistics

Statistical analyses were performed with SPSS, version 14. All results are reported as mean \pm 1 SD unless stated otherwise and were considered to be significant at $P < 0.05$. For all parametric tests, assumption of normality and homogeneity of variances were evaluated using the Kolmogorov-Smirnov test and Levene's test, respectively (Zar 1999). As a measure of structural size, we derived scores of the first principal component (PC1) from a factor analysis based on four external measurements: the lengths of tarsus, total head (i.e., including bill), maximum wing chord, and keel (measured from the anterior tip of the carina to the end of the sternum, at the transition with the abdominal cavity). Tarsus was measured with calipers to the nearest 0.1 mm; all other variables were measured with a ruler at 1-mm accuracy. Variables had a similar factor loading (0.72–0.94) on PC1, which explained 73% of the total variance. We used ANOVA to test for mean differences among sex and GLM, with PC1 included as covariate.

Differences in estimated dilution space with time after isotope injection were compared by a repeated-measures ANOVA. Two-tailed paired t -tests were used to compare means of TBW_d and TBW_c . We used estimated TBW and other predictor var-

Table 1: Whole-body composition by carcass analysis

	Total (<i>n</i> = 21)	Males (<i>n</i> = 11)	Range	Females (<i>n</i> = 10)	Range
BM (g)	1,995 ± 241*	2,104 ± 183	1,893–2,515	1,876 ± 248	1,479–2,185
TBW _c (g)	1,121 ± 152*	1,213 ± 112	1,079–1,457	1,020 ± 125	799–1,209
FFM (g)	1,773 ± 224*	1,907 ± 159	1,729–2,273	1,625 ± 193	1,263–1,901
FM (g)	222.5 ± 110.7	196.4 ± 99.0	37.1–330.6	251.2 ± 120.9	95.6–539.6
TBW _c : BM (%)	56.2 ± 3.9 ^a	57.7 ± 3.7	52.9–62.9	54.6 ± 3.5	46.9–59.3
TBW _c : FFM (%)	63.2 ± 1.1 ^a	63.5 ± 1.3	61.8–65.4	62.8 ± .8	61.1–63.8
FM : BM (%)	11.1 ± 5.1	9.2 ± 4.5	2.0–15.3	13.1 ± 5.2	6.5–24.7

Note. Given are body mass (BM), total body water from carcass analysis (TBW_c), fat-free mass (FFM), fat mass (FM), and fractions (%) of water and fat content for sexes combined and males and females separately.

^a Including fresh plumage mass in BM; if plumage is excluded from BM, the fractions are TBW_c : BM = 60.6% ± 4.1% and TBW_c : FFM = 68.8% ± 0.8%.

* Significant differences between sexes ($P < 0.05$).

ables (see below) to predict FM and FFM by two approaches: (1) multiple regression analyses and (2) assuming a constant FFM hydration.

1. We applied a stepwise backward elimination procedure in the multiple regression analyses. Starting with the nondestructively obtained variables tarsus, head, wing, keel, BM, and sex as a basic model, we extended the set of predictor variables and included either TBW_d and dry BM or TBW_c and dry BM to investigate whether FM predictions could be improved. Finally, we took the set of predictor variables from the basic model and included additionally abdominal fat fresh mass (i.e., of the dissected fat pad, not chemically extracted fat). Before we included abdominal fat in multiple regressions, we tested whether a curvilinear relationship between FM and abdominal fat would be more appropriate than a linear fit (Piersma 1984). Although the coefficient of determination increased slightly from $r^2 = 0.90$ to $r^2 = 0.92$, adding a quadratic term did not significantly improve a linear fit of FM to abdominal fat ($P = 0.06$).

We used double cross-validation to evaluate the robustness and replicability of regression equations following the procedure described by Guan et al. (2004). Briefly, subjects from the original data set were randomly assigned to two groups, equal or similar in number and sex ratio of subjects. The statistically significant predictor variables derived from the original full data set were applied in both subgroups to develop predictive equations and derive coefficients of determination (r_{11}^2 and r_{22}^2 , where the first subscript number refers to subsample's data and the second subscript number to subsample's regression coefficients). Standardized regression coefficients and Z scores of predictor variables and of the response variable were used in all cross-validation procedures. The predictors' regression coefficients were crossed over the two subsamples to produce equations and coefficients of determination (r_{12}^2 and r_{21}^2) from actual group data using the regression coefficients from the other group for the predictions. Using this double-cross procedure, we calculated two shrinkage values: $r_{11}^2 - r_{12}^2$ and $r_{22}^2 - r_{21}^2$. The more closely the shrinkage estimate approaches 0, the greater the degree of stability is across subsamples. Furthermore, two invariance coefficients were derived by correlating

the predicted values of subsample 1 with the predicted values of subsample 1 using the regression coefficients of subsample 2 (r_{11-12}) and vice versa (r_{22-21}). As these invariance coefficients approach 1, more confidence can be obtained in the replicability of the results.

2. As an alternative to calibrated regression equations and assuming a constant water content in the FFM ($H_2O_{FFM} = TBW : FFM = \text{constant}$), we can infer the individual FFM and FM from the following equations:

$$FFM = \frac{TBW}{H_2O_{FFM}}, \quad (2)$$

$$FM = BM - FFM. \quad (3)$$

We will refer to this approach as the Pace and Rathbun (1945) approach (sensu Mata et al. 2006).

Results

Body Composition by Carcass Analysis

Carcass analyses are listed in Table 1. Animals in this study covered a broad scale of BM and composition, ranging twofold in BM and from 2% to 25% in lipid content. Males were larger than females according to PC1, the first principal component from a factor analysis including tarsus, wing, skull, and keel length ($F_{1,19} = 31.18$, $P < 0.001$). Significant differences between sexes were also found for BM, TBW, and FFM. However, these were caused by the sex-related differences in structural size. Thus, when sex was tested together with PC1 in one analysis to explain differences in BM, TBW, and FFM, only PC1 explained a significant part ($P < 0.05$ in all models), whereas variation due to sex became nonsignificant. Females in our sample tended to have higher fat loads (FM : BM, $F_{1,19} = 3.39$, $P = 0.08$).

TBW Measured by Deuterium Dilution and the Effect of Equilibration Time

Within-individual variation in deuterium isotope enrichment occurred mainly between the first two sampling events and was independent of the size of the individual body water pool (Fig. 1). However, a pronounced increase over this period was restricted to a few individuals only, and overall changes between sampling times were too low to be significant (repeated-measures ANOVA, $F_{2,30} = 1.894$, $P = 0.168$).

At all sampling intervals, carcass body water was significantly overestimated by the deuterium dilution space (paired t -test, $P < 0.001$), ranging from average values of 9.2% at 45 min to 7.1% at 90 min (Table 2). This overestimate was consistent over the range of TBW_c studied ($P > 0.05$ for all regression models at the various sampling times). The accuracy in predicting TBW from deuterium dilution was, on average, slightly better at 90 and 180 min than at 45 min, but this difference was not significant (repeated-measures ANOVA, $F_{2,30} = 2.130$, $P = 0.136$). Variance of the ratio of $TBW_d : TBW_c$ was significantly different between sampling events (Levene's test, $F_{2,49} = 4.473$, $P = 0.016$). The error in the deviation of TBW_d from TBW_c was twice as high at 45 min compared to 90 and 180 min after dose administration (Table 2), leading to a reduced precision of TBW predictions from isotope measurements at this early sampling stage. The following regression equations were derived to predict TBW_c from TBW_d at 90 min ($n = 20$) and 180 min ($n = 16$), respectively:

$$TBW_c = 96.034 + 0.852 \times TBW_d, \quad (4)$$

$$TBW_c = 94.544 + 0.848 \times TBW_d. \quad (5)$$

When using deuterium dilution space as a predictor variable to estimate body composition, we employed TBW_d values measured at 90 min.

Estimates of Body Composition by Lethal and Nonlethal Methods

TBW determined from either carcass analysis or isotope dilution was a very strong single predictor for FFM, explaining 98%–99% of the variation in FFM (Fig. 2). Table 3 compares the outcome of multiple regression analyses using TBW measured by deuterium isotope dilution and carcass analysis and further predictors related to different levels of invasiveness. The errors in the prediction of FM and FFM (calculated as deviations of predicted from observed values) following the Pace and Rathbun (1945) approach are also listed in Table 3. For the latter approach, we applied individual estimates of TBW from the established relationship between TBW_c and TBW_d (measured at 90 min equilibrium time) and a H_2O_{FFM} of 63.2% (i.e., the average from our sample). We presented only predictive equations for FM because the absolute error of prediction was the same for FM and FFM, regardless of which component was taken as a response variable. This was due to the same

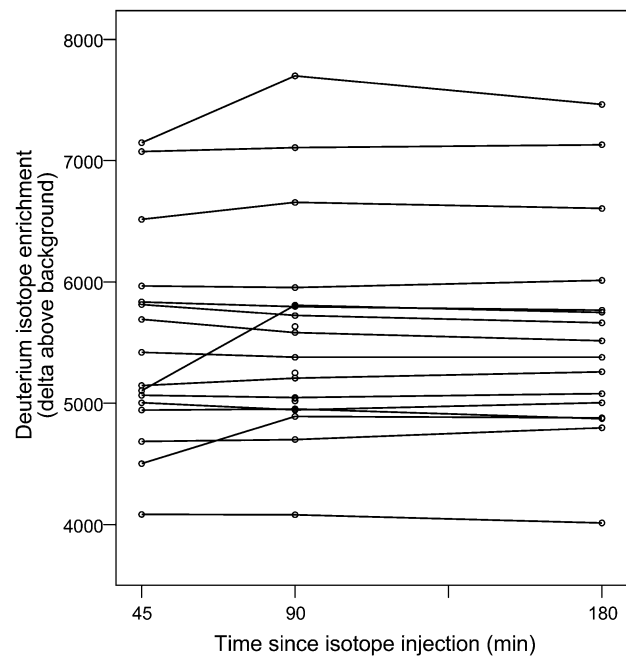


Figure 1. Deuterium isotope enrichment determined for 16 animals sampled at 45, 90, and 180 min after isotope injection and another four animals sampled at 90 min after dose administration.

significant predictors (models 1–3) for both FM and FFM and the fact that they add up exactly to BM. Thus, FFM was calculated as $FFM = BM - FM$. Also, we were mainly interested in the variation of FM (the relatively smaller of both components) and to what extent it can be accounted for by the various regression models.

Much of the variation in FM was unaccounted for by model 1 based on BM and external body measurements. Furthermore, relatively strong r^2 shrinkage and low invariance coefficients indicate lower replicability of the prediction when applied to different subsamples compared to the other models. FM was not significantly related to any of the potential predictors offered to model 1 separately. Only the combination of BM and a structural measurement (keel) revealed a significant relationship with FM. The combined variables dry BM (i.e., $BM - TBW$) and TBW determined from isotope dilution (model 2) or carcass analysis (model 3) both explained a large part of the variation in FM (92% and 97%, respectively). Moreover, the results from a cross-validation showed good replicability of the respective equations. When dry BM was replaced by BM in models 2 and 3, errors of prediction and r^2 were the same. However, such alternative models suffered notably from multicollinearity of the predictors (see VIF in Table 3), making them less robust. Abdominal fat fresh mass was highly correlated with total FM, and if it was the single predictor in the model, it accounted for 90% of the variation in FM. The variation increased slightly to 93% when head length was added to this model.

Table 2: Deuterium dilution space (TBW_d) measured at different sampling intervals and in relation to total body water from carcass analysis (TBW_c)

	45 min	90 min	180 min
	<i>n</i> = 16	<i>n</i> = 16 ^a	<i>n</i> = 20
TBW _d (g)	1,198 ± 185	1,179 ± 187	1,190 ± 169
TBW _d : TBW _c	1.092 ± .054	1.073 ± .027	1.071 ± .026
<i>r</i> ² _{TBW_c-TBW_d} (SEE in g)	.90 (51)	.98 (22)	.98 (23)
			<i>n</i> = 16
TBW _d (g)			1,182 ± 185
TBW _d : TBW _c			1.076 ± .024
<i>r</i> ² _{TBW_c-TBW_d} (SEE in g)			.98 (20)

Note. Data represent mean ± SD. Also given are coefficients of determination (*r*²) and the standard errors of the estimates (SEEs; i.e., the root mean square errors) from linear regressions of TBW_c versus TBW_d (*P* < 0.001 for all regressions).

^a Includes only birds that were also measured at 45 and 180 min.

Discussion

We found a strong relationship between TBW measured by carcass desiccation and by deuterium isotope dilution in barnacle geese, which was consistent over a large range of body composition. With knowledge of this relationship and of the minimum equilibrium time needed for the isotope dose to mix completely with the body water pool, TBW can be very accurately predicted by deuterium isotope dilution as a nonlethal method. Furthermore, an accurate estimate of TBW was a strong predictor of FFM and, in combination with BM, FM. However, the accuracy of the estimates of FFM and FM for additional samples on the basis of TBW measured isotopically or by desiccation largely depends on the variation of the relative water content in the FFM.

Body Composition by Carcass Analysis

The dissected barnacle geese showed pronounced sexual differences in structural size and, related to this, differences in BM, FFM, and TBW. Higher fat loads in females may be expected in geese, in particular during the prebreeding phase, in anticipation of egg production and incubation (Raveling 1979).

FFM Hydration in Birds: How Variable Is It?

Whenever TBW, or an estimate thereof, is used to predict FFM, the variation of H₂O_{FFM} is of crucial relevance because it comprises the other major error source in addition to the error of the TBW estimate. Wang et al. (1999) reviewed this issue for adult mammals and concluded that species share a relatively constant H₂O_{FFM} in spite of differences in BM ranging by a factor of 10⁴. The relatively higher water content in the lean body component of the young growing organism until it reaches chemical maturity is well known (e.g., for mammals, Arnould et al. 1996; for birds, Bech and Østnes 1999), and thus we will restrict ourselves to a discussion of mature birds.

When we compare results of H₂O_{FFM} among studies, first of all, our attention has to be on possible differences in methodologies and definitions applied in these studies. Researchers often include water adsorbed to the feathers in the amount of TBW, which may amount to ca. 1%–2.5% of TBW (Crum et

al. 1985; Mata et al. 2006). We intentionally did not do so because this “external water” is not in exchange with the body water pool estimated by isotope dilution (Crum et al. 1985). Further, FFM may be differently defined among studies, excluding, for instance, plumage (e.g., Boos et al. 2000; Mata et al. 2006) or bones and plumage (e.g., Groscolas et al. 1991) from the FFM component. Consequently, estimates of H₂O_{FFM} from those studies (71%–73%) are necessarily considerably higher than the value reported here.

In Table 4, we compiled data on H₂O_{FFM} reported or calculated from studies of waterfowl carcass analyses using comparable methods and definitions as applied in this study. H₂O_{FFM} can be reasonably stable over different seasonal and/or physiological stages within a given study. As in our study, no sex-related differences are indicated. Variation between studies can, however, be considerable.

Compared to those of other studies, our values (mean = 63.2%) are at the lower range of H₂O_{FFM} values reported so far. Although the geese had access to water during the captive period until 4–6 h before termination of the experiment, due to

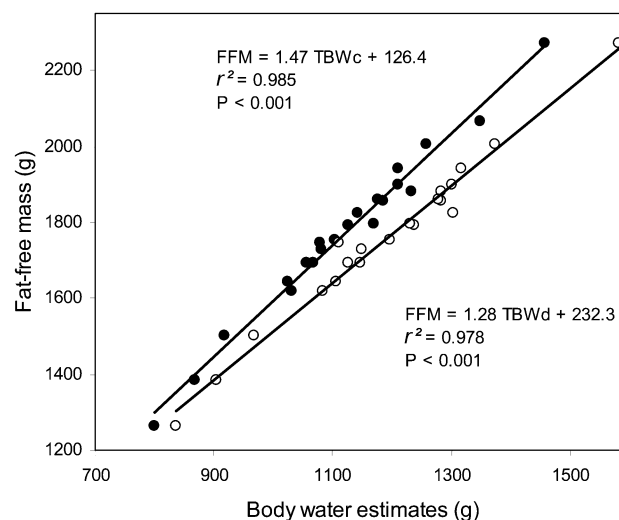


Figure 2. Relationships between fat-free mass and body water determined by carcass analysis (filled circles; *n* = 21) and deuterium dilution (open circles; *n* = 20).

Table 3: Predictive equations for total fat mass (FM; g) based on nonlethal and lethal measurements in 21 barnacle geese

Multiple regression models							Shrinkage			Invariance Coefficients	
	r^2	SEE	Absolute Error FM and FFM (g)	Error FFM (%)	Error FM (%)	VIF			r_{11-12}	r_{22-21}	
							$r_{11}^2 - r_{12}^2$	$r_{22}^2 - r_{21}^2$			
Nonlethal:											
1. FM = 368.7 + .329 × BM − 6.997 × keel	.418	89.1	69.9 ± 44.8	4.0 ± 2.8	37.3 ± 24.9	1.42	.19	.11	.84	.74	
2. FM = −200.6 − .266 × TBW _d + .936 × dry BM	.918	33.7	21.3 ± 23.3	1.2 ± 1.4	10.1 ± 10.1	1.15 ^a	.02	.01	.99	.99	
Lethal:											
3. FM = − 57.48 − .418 × TBW _c + .856 × dry BM	.966	21.6	16.0 ± 12.3	.9 ± .8	10.6 ± 11.4	1.18 ^b	.14	.12	.93	.94	
4. FM = − 382.7 + 4.492 × F _{abd} + 5.223 × head	.931	30.1	24.9 ± 13.8	1.4 ± .8	16.0 ± 18.4	1.01	.01	.01	1.00	1.00	
Pace and Rathbun (1945) approach											
			22.1 ± 26.0	1.3 ± 1.5	12.7 ± 17.1						

Note. TBW_d = deuterium dilution space; TBW_c = total body water from the carcass analyses. $n = 20$ if TBW_d is used in the equation. Modeling started with noninvasively measurable predictors in model 1: tarsus, head, wing, keel, body mass (BM), and sex. This set of potential predictors was expanded by TBW_d and dry BM (i.e., BM – TBW_d) in model 2 and by TBW_c and dry BM (i.e., BM – TBW_c) in model 3. For model 4, the same predictors were applied as for model 1, with the addition of abdominal fat fresh mass (F_{abd}). Shown are the final models containing only significant predictor variables derived by stepwise backward multiple regressions. For all models, $P < 0.001$ (except for model 1, $P = 0.008$). Summary statistics include coefficients of determination (r^2), standard error of the estimate (SEE), absolute and relative error of predicted FM and fat-free mass (FFM) calculated as deviations of predicted from observed values (mean \pm SD), variance inflation factor of predictors (VIF), and statistics from a cross-validation procedure (see “Methods” for details). FFM was calculated as BM – FM (see text). Outcomes of the Pace and Rathbun (1945) approach are given in the last row ($n = 20$).

^a When dry BM is replaced by BM in the model, VIF = 4.51.

^b When dry BM is replaced by BM in the model, VIF = 3.68.

general capture stress they may have not made sufficient use of it and may have experienced a certain degree of dehydration. Birds can tolerate notable water losses under restrained conditions. For instance, Davidson (1984) noted a decrease of $\text{H}_2\text{O}_{\text{FFM}}$ of 0.8%–1% per hour during the first 4 h after capture in knots (*Calidris canutus*) and dunlins (*Calidris alpina*), that is, from 66.7% to 63.5% and from 65.8% to 61.8%, respectively. Interestingly, after the first 4 h, dunlins appeared fully to compensate further water loss by metabolically produced water, whereas in knots, dehydration continued (at a lower rate) until 60.9% at 24 h after capture.

We conclude that both methodological factors and tolerance for a (temporarily) negative water balance in birds may contribute to the considerable variation in $\text{H}_2\text{O}_{\text{FFM}}$ reported among studies. Nevertheless, homeostasis is crucial for organismal functioning. Therefore, under unrestrained circumstances, birds will probably strive to avoid dehydration and keep their water balance within small margins. Currently, it is not possible to rule out whether and to what extent variation in $\text{H}_2\text{O}_{\text{FFM}}$ reflects truly species-specific diversity or varying sampling procedures and other methodological differences. Future applications of the isotope dilution method to estimate body composition would benefit from a better understanding of general patterns of variation in $\text{H}_2\text{O}_{\text{FFM}}$ and how it may be related to species and/or physiological state.

TBW Measured by Deuterium Dilution and the Effect of Equilibration Time

In this study, TBW_d overestimated TBW_c by 7.1% (at 90 min equilibrium time) consistently over a large range of TBW_c and can thus be corrected according to the values given here. Speakman et al. (2001; Table 3) calculated an average of 4.7% by which actual TBW was overestimated by hydrogen isotope dilution in birds, based on nine studies on four species. Additional studies using hydrogen isotopes and the plateau approach reported 8.1% for the chicken *Gallus gallus* (Mata et al. 2006), 8.4% for glaucous-winged gull *Larus glaucescens* nestlings (Hughes et al. 1987), and 3.3% for the glaucous gull *Larus hyperboreus* (Shaffer et al. 2006). However, at least part of the variation among studies is due to methodological factors. For instance, the study by Degen et al. (1981), which was included in the average calculated by Speakman et al. (2001), as well as those by Hughes et al. (1987) and Shaffer et al. (2006) included water adsorbed to feathers in TBW_c. This plumage water cannot be accounted for by isotope dilution. As a consequence, the difference between TBW_d and TBW_c becomes smaller. Thus, an average value by which carcass TBW (excluding plumage water) is overestimated by the hydrogen dilution space in birds certainly exceeds the 4.7% given by Speakman et al. (2001).

Our results indicate that mixing of marker solution and body water was not completed after 45 min. Average levels and var-

Table 4: Data on the relative water content in the fat-free mass (H_2O_{FFM}) in adult waterfowl

Species and Period of Collection	H_2O_{FFM} (%)				Reference
	Females	<i>n</i>	Males	<i>n</i>	
Barnacle goose (<i>Branta leucopsis</i>)					This study
Winter–spring	62.8 (.2)	10	63.5 (.4)	11	
Black duck (<i>Anas rubripes</i>)					Reinecke et al. 1982
Prelay	64.5	4			
Laying	64.3	12			
Postlay	65.0	16			
Moult	63.8	3			
Autumn	63.2	7			
Winter	60.3	11			
Mean	63.5				
Brent goose (<i>Branta bernicla</i>)					Korte 1988
Winter–spring	69.0 (.6)	14	68.4 (.5)	21	
Canada goose (<i>Branta canadensis</i>)					Raveling 1979
Autumn migration	65.2	6	63.5	9	
Midwinter	62.6	5	62.9	10	
Spring migration	65.7	11	65.8	5	
Prelay	63.4	4	65.4	5	
Midincubation	65.4	2	66.5	1	
Hatch day	66.1	9	65.2	6	
Early moult	68.7	8	68.1	9	
Midmoult	67.5	2			
Mean	65.6		65.3		
Common eider (<i>Somateria mollissima</i>)					Parker and Holm 1990
2–3 wk prelay	63.6	4			
Prelay breeders	64.4	7			
Prelay nonbreeders	66.5	2			
Postlay	63.9	7			
Hatch day	64.6	8			
Mean	64.6				
Lesser scaup (<i>Aythya affinis</i>)					Austin and Fredrickson 1987
Premoult	73.3	21			
Moult	70.8	24			
Postmoult	71.5	8			
Migratory	70.8	32			
Mean	71.6				
Lesser snow goose (<i>Anser c. caerulescens</i>)					Campbell and Leatherland 1980
Through the year	69.3 (.06) ^{a,b}				
Mean of all studies	66.6 (1.2) ^c	7			

Note. In most cases, H_2O_{FFM} was calculated from mean values of body mass, fat mass, and total body water (TBW; except from individual values in Korte 1988; Campbell and Leatherland [1980] gave mean values directly). We used only samples (means) where the same birds were analyzed for all of those body components. *n* = sample size. Standard errors are shown in parentheses.

^a TBW component included water adsorbed to feathers. Reference was unclear about the actual sample size related to the reported mean and standard error of 0.693 ± 0.06 . Translated into percentage, this standard error would equal a questionable 0.0006%. We suspect that 0.06 is the correct value.

^b Sexes were pooled.

^c Mean of studies' means; for studies where data for both sexes were available, the average was used.

iability were the same at 180 and 90 min, indicating that 90 min is sufficient time to allow for adequate equilibration. Apparently, compared to an earlier estimate of about 4 h for this species (Nolet et al. 1992), equilibration time can be considerably reduced without compromising accuracy of the TBW estimate, thereby reducing disturbance to the animal.

Estimates of Body Composition by Lethal and Nonlethal Methods

Regression models to predict body composition based on BM in combination with other external morphological parameters performed poorly in this study of the barnacle goose (Table 3;

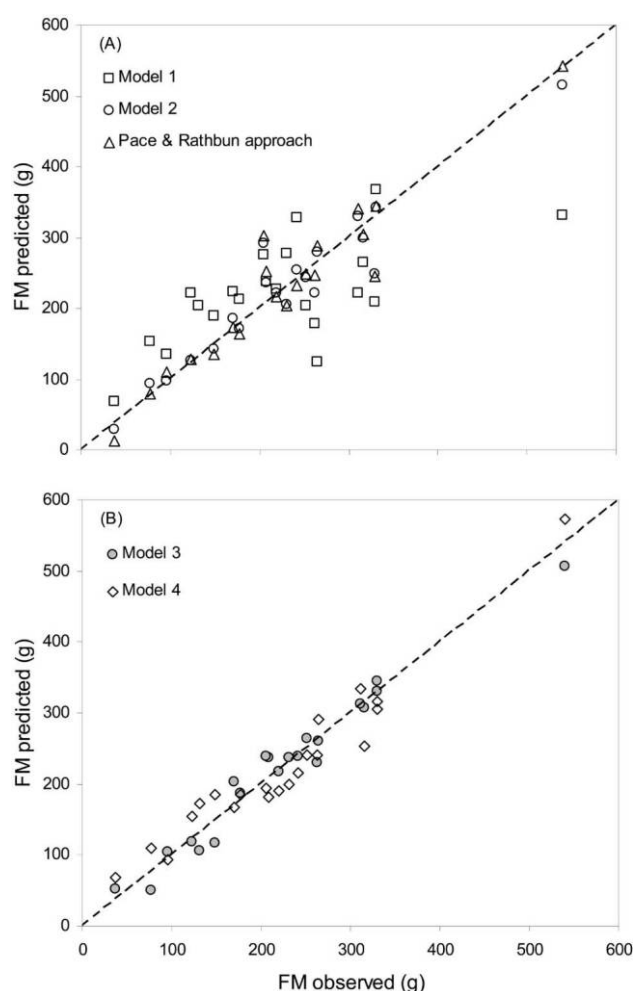


Figure 3. Relationships between fat mass determined by carcass analysis and predictions of models presented in Table 3. A, Nonlethal methods; B, lethal methods. Dashed line = relationship of $Y = X$.

Fig. 3). BM alone was unable to explain a significant part in FM variation. In contrast, Skagen et al. (1993) found in two sandpiper species 76% and 89% of the variance in FM explained by BM. There are two main reasons why BM alone may be a weak predictor for FM. First, changes in BM can involve, in addition to fat, appreciable amounts of protein, and the relative contribution of both components may vary over periods of mass change. Studies of geese provide good examples (Newton 1977; Raveling 1979; Prop and Spaans 2004) where such changes were related to seasonal and physiological stages such as migration, reproduction, and moult. Second, study subjects usually differ in structural size, and such differences are generally unrelated to lipid contents, although some relationship may occur when fat is deposited in the bone marrow, which can reach significant levels in waterfowl (Hutchinson and Owen 1984). Additional incorporation of structural measurements can improve the predictive power of the model but accounts for only part of the overall structural size differences. Because

FFM includes virtually all structural mass and, at the same time, nearly all water, a measure of TBW better accounts for differences due to structural mass than do most morphological measurements. Indeed, variation in TBW determined from carcass analysis or isotope dilution both explained a large part of the variation in FFM and, consequently, FM.

Abdominal fat fresh mass was highly correlated with total FM and, if the carcass is available, allows simple means of fat prediction without dissecting the entire specimen. Model 1 represented the least invasive of the methods tested in Table 3. However, the error of prediction was highest, equating to 37% and 4.0% of actual FM and FFM, respectively. Using estimates of TBW from deuterium isotope dilution and either a regression or the Pace and Rathbun (1945) approach as alternative non-destructive methods reduced the relative error of the FM and FFM estimate to 10%–13% and 1.2%–1.3%, respectively.

Differences in the accuracy of a regression and the Pace and Rathbun (1945) approach depend on how much individual animals in the study sample deviate from the assumption of a constant FFM hydration. Variation in H_2O_{FFM} was low in our sample (Table 1), resulting in a similar accuracy of both approaches (Table 3; Fig. 3). Irrespective of which approach is applied, prediction accuracy for a new sample will be reduced if H_2O_{FFM} differs between the calibration sample and a new sample. A direct measurement of H_2O_{FFM} in a subsample of the study population is always advisable to ensure confidence about resulting predictions. If this is not possible, we suggest applying an average value of 66.6% for studies on adult waterfowl, as calculated from the species-specific studies listed in Table 4, when using the Pace and Rathbun (1945) approach.

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Literature Cited

- Arnould J.P.Y., I.L. Boyd, and J.R. Speakman. 1996. Measuring the body composition of Antarctic fur seals (*Arctocephalus gazella*): validation of hydrogen isotope dilution. *Physiol Zool* 69:93–116.
- Austin J.E. and L.H. Fredrickson. 1987. Body and organ mass and body composition of postbreeding female lesser scaup. *Auk* 104:694–699.
- Bech C. and J.E. Østnes. 1999. Influence of body composition

- on the metabolic rate of nestling European shags (*Phalacrocorax aristotelis*). *J Comp Physiol B* 169:263–270.
- Blem C.R. 1990. Avian energy storage. Pp. 59–113 in D.M. Power, ed. *Current Ornithology*. Plenum, New York.
- Boos M., T. Zorn, A. Koch, Y. Le Maho, and J.P. Robin. 2000. Determining body fuels of wintering mallards. *C R Acad Sci III Life Sci* 323:183–193.
- Brown M.E. 1996. Assessing body condition in birds. Pp. 67–135 in V. Nolan and E.D. Ketterson, eds. *Current Ornithology*. Plenum, New York.
- Campbell R.R. and J.F. Leatherland. 1980. Estimating body protein and fat from water content in lesser snow geese. *J Wildl Manag* 44:438–446.
- Carey C., ed. 1996. *Avian Energetics and Nutritional Ecology*. Chapman & Hall, New York.
- Cherel Y., J.P. Robin, A. Heitz, C. Calgari, and Y. Le Maho. 1992. Relationships between lipid availability and protein utilization during prolonged fasting. *J Comp Physiol B* 162:305–313.
- Crum B.G., J.B. Williams, and K.A. Nagy. 1985. Can tritiated water-dilution space accurately predict total body water in chukar partridges? *J Appl Physiol* 59:1383–1388.
- Culebras J.M. and F.D. Moore. 1977. Total body water and the exchangeable hydrogen. I. Theoretical calculation of non-aqueous exchangeable hydrogen in man. *Am J Physiol* 232:R54–R59.
- Davidson N.C. 1984. Changes in the condition of dunlins and knots during short-term captivity. *Can J Zool* 62:1724–1731.
- Degen A.A., B. Pinshow, P.U. Alkon, and H. Arnon. 1981. Tritiated water for estimating total body water and water turnover rate in birds. *J Appl Physiol* 51:1183–1188.
- Ellis H.I. and J.R. Jehl. 1991. Total body water and body composition in phalaropes and other birds. *Physiol Zool* 64:973–984.
- Gehre M., H. Geilmann, J. Richter, R.A. Werner, and W.A. Brand. 2004. Continuous flow $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ analysis of water samples with dual inlet precision. *Rapid Commun Mass Spectrom* 18:2650–2660.
- Gessaman J.A. 1999. Evaluation of some nonlethal methods of estimating avian body fat and lean mass. Pp. 2–16 in N. Adams and R. Slotow, eds. *Proceedings of the 22nd International Ornithological Congress*. University of Natal Press, Durban.
- Groscolas R., L. Schreiber, and F. Morin. 1991. The use of tritiated water to determine protein and lipid utilization in fasting birds: a validation-study in incubating great-winged petrels, *Pterodroma macroptera*. *Physiol Zool* 64:1217–1233.
- Guan J., P. Xiang, and X.D. Keating. 2004. Evaluating the replicability of sample results: a tutorial of double cross-validation methods. *Meas Phys Educ Exercise Sci* 8:227–241.
- Houston A.I., P.A. Stephens, I.L. Boyd, K.C. Harding, and J.M. McNamara. 2007. Capital or income breeding? a theoretical model of female reproductive strategies. *Behav Ecol* 18:241–250.
- Hughes M.R., J.R. Roberts, and B.R. Thomas. 1987. Total body water and its turnover in free-living nestling glaucous-winged gulls with a comparison of body water and water flux in avian species with and without salt glands. *Physiol Zool* 60:481–491.
- Hutchinson A.E. and R.B. Owen. 1984. Bone-marrow fat in waterfowl. *J Wildl Manag* 48:585–591.
- Jamieson S.E., H.G. Gilchrist, F.R. Merkel, K. Falk, and A.W. Diamond. 2006. An evaluation of methods used to estimate carcass composition of common eiders *Somateria mollissima*. *Wildl Biol* 12:219–226.
- Korte H. 1988. *Measuring Condition in Brent Geese Branta b. bernicla*. MS thesis. University of Groningen, Groningen.
- Mata A.J., M. Caloin, J.P. Robin, and Y. Le Maho. 2006. Reliability in estimates of body composition of birds: oxygen-18 versus deuterium dilution. *Physiol Biochem Zool* 79:202–209.
- McNab B.K. 2002. *The Physiological Ecology of Vertebrates: A View from Energetics*. Cornell University Press, Ithaca, NY.
- Miller M.R. 1989. Estimating carcass fat and protein in northern pintails during the nonbreeding season. *J Wildl Manag* 53:123–129.
- Newton I. 1977. Timing and success of breeding in tundra-nesting geese. Pp. 113–126 in B. Stonehouse and C. Perrins, eds. *Evolutionary Ecology*. Macmillan, London.
- Nolet B.A., P.J. Butler, D. Masman, and A.J. Woakes. 1992. Estimation of daily energy expenditure from heart rate and doubly labeled water in exercising geese. *Physiol Zool* 65:1188–1216.
- Odum E.P., D.T. Rogers, and D.L. Hicks. 1964. Homeostasis of the nonfat components of migrating birds. *Science* 143:1037–1039.
- Olsen J., I. Seierstad, B. Vinther, S. Johnsen, and J. Heinemeier. 2006. Memory effect in deuterium analysis by continuous flow isotope ratio measurement. *Int J Mass Spectrom* 254:44–52.
- Pace N. and E.N. Rathbun. 1945. Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. *J Biol Chem* 158:685–691.
- Parker H. and H. Holm. 1990. Patterns of nutrient and energy expenditure in female common eiders nesting in the High Arctic. *Auk* 107:660–668.
- Piersma T. 1984. Estimating energy reserves of great crested grebes *Podiceps cristatus* on the basis of body dimensions. *Ardea* 72:119–126.
- Prop J. and B. Spaans. 2004. Body stores in pre-migratory brent geese: the consequence of habitat choice on protein deposition. Pp. 71–85 in J. Prop, ed. *Food Finding: On the Trail to Successful Reproduction in Migratory Geese*. PhD thesis. University of Groningen, Groningen.
- Raveling D.G. 1979. The annual cycle of body composition of Canada geese with special reference to control of reproduction. *Auk* 96:234–252.
- Reinecke K.J., T.L. Stone, and R.B. Owen. 1982. Seasonal carcass composition and energy balance of female black ducks in Maine. *Condor* 84:420–426.

- Schoeller D.A., E. Van Santen, D.W. Peterson, W. Dietz, J. Jaspán, and P.D. Klein. 1980. Total body water measurement in humans with ^{18}O and ^2H labeled water. *Am J Clin Nutr* 33:2686–2693.
- Shaffer S.A., G.W. Gabrielsen, J. Verreault, and D.P. Costa. 2006. Validation of water flux and body composition in glaucous gulls (*Larus hyperboreus*). *Physiol Biochem Zool* 79:836–845.
- Skagen S.K., F.L. Knopf, and B.S. Cade. 1993. Estimation of lipids and lean mass of migrating sandpipers. *Condor* 95: 944–956.
- Speakman J.R., ed. 2001. *Body Composition Analysis of Animals: A Handbook of Non-Destructive Methods*. Cambridge University Press, Cambridge.
- Speakman J.R., G.H. Visser, S. Ward, and E. Krol. 2001. The isotope dilution method for the evaluation of body composition. Pp. 56–98 in J.R. Speakman, ed. *Body Composition Analysis of Animals: A Handbook of Non-Destructive Methods*. Cambridge University Press, Cambridge.
- Stevenson R.D. and W.A. Woods. 2006. Condition indices for conservation: new uses for evolving tools. *Integr Comp Biol* 46:1169–1190.
- Thomas V.G., S.K. Mainguy, and J.P. Prevett. 1983. Predicting fat content of geese from abdominal fat weight. *J Wildl Manag* 47:1115–1119.
- Wang Z.M., P. Deurenberg, A. Pietrobelli, R.N. Baumgartner, and S.B. Heymsfield. 1999. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr* 69:833–841.
- Zar J.H. 1999. *Biostatistical Analysis*. 4th ed. Prentice Hall, Upper Saddle River, NJ.